REMARKS

The Office Action dated December 22, 2000 has been received and carefully noted. The above amendments and the following remarks are submitted as a full and complete response thereto.

Claims 1-17, 19, 20, 46 and 47 are all the claims before the Examiner for further consideration. New claims 48-55 have been added.

The specification has been amended to correct a typographical error with respect to the designation for the pop2135 strain of bacteria. Support for the correct numerical designation can be found in the Examples (e.g., page 21; Recombinant expression of the sbsA gene in E. coli).

I. Status of the Claims

Claims 1-17, 19, 20, 46 and 47 have been amended to more particularly point out and distinctly claim the present invention.

Claim 1 has been amended to recite: proper Markush group language; the term "protein" throughout the claim; and the nucleic acid of (iii) comprising at least one of (i) or (ii).

Claim 2 is now written in proper dependent claim format and places a limitation for a species on the gram-negative host cell.

Claim 3 is now written in proper dependent claim format and recites the term "protein" to bring all of the claims into uniformity with respect to this term.

Claim 4 is now written in proper dependent claim format and to indicate that the S-layer protein contains "one or more insertions". Various insertions having favorable properties are described in the specification, and a high functional density of S-layer proteins can be achieved by adding a plurality of insertions.

Claim 5 is now written in proper dependent claim format and to recite proper Markush group language.

Claims 6-7 are now written in proper dependent claim format.

Claims 8-10 are now written in proper dependent claim format and to replace the phrase "such as" with "comprising".

Claims 11-13 are now written in proper dependent claim format.

Claim 14 is now written in proper dependent claim format and to recite that the nucleotide sequence of c) is al least one of a) or b).

Claim 15 now recites proper Markush group language and the nucleic acid of (iii) is limited to at least one of (ii) or (iii).

Claim 16 has been amended to recite the claimed nucleic acid with greater clarity.

Claim 17 has been amended to recite the claimed vector with greater clarity.

Claim 19 has been amended to recite that the cell is transformed with a nucleic acid molecule or a vector, and to correct the improper dependency of the claim.

Claim 20 has been amended to correct the improper dependency of the claim.

Claims 46 and 47 have been amended to recite the properties for the transformed cell with greater clarity.

New claim 48 finds support, *inter alia*, in the canceled subject matter of claim 1, i.e., the negative proviso.

New claim 49 recites the stringent hybridization conditions for the process of claim 1 (page 3, lines 21-26).

New claim 50, which depends from claim 49, recites a further limitation for the stringency condition.

New claim 51 finds support, *inter alia*, in the canceled subject matter of claim 15, i.e., the negative proviso.

New claim 52 recites the stringent hybridization conditions for the nucleic acid of claim 15.

New claim 53, which depends from claim 52, recites a further limitation for the stringency condition.

New claim 54 finds support, *inter alia*, in the canceled subject matter of original claim 19, i.e., the limitation to an E. coli cell.

New claim 55 finds support, *inter alia*, in the canceled subject matter of original claim 7, i.e., the limitation to herpes virus 6 or FMDV.

No new matter has been added. Claims 1-17, 19, 20 and 46-55 are respectfully submitted for consideration.

II. Response to Objection to Claims 19 and 20

Claims 19 and 20 have been amended to incorporate the subject matter of cancelled claim 18, and to correct their improper dependency from claim 18.

III. Response to Rejection of Claims 1-17, 19, 20 46 and 47 under 35 U.S.C. 112, second paragraph

Claims 1-17, 19, 20 46 and 47 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite.

A. Claim 1

Claim 1 recites an alternative expression for two limitations covering two different embodiments, i.e. the embodiment of "(i)" is not the same as the embodiment of "(ii)".

Applicants traverse and submit that the claim as amended to recite "at least one of the nucleic acids of (i) or (ii)", does not change the scope of the claimed subject matter.

B. Claim 14

Claim 14 recites an alternative expression for three limitations covering three different embodiments, i.e. the embodiment of "(a)" is not the same as the embodiment of "(b)" and the embodiment of "(a)" is not the same as the embodiment of "(c)".

For the transition between elements b) and c), Applicants have deleted "and/or" and inserted -- or --. For element c), the phrase "and/or" has been replaced with the phrase "at least one nucleotide sequence of (a) or (b)".

C. Claim 15

Claim 15 recites an alternative expression for two limitations covering two different embodiments, i.e. the elements in "(i)" are not the same as the elements in "(ii)".

In element (iii), Applicants have deleted the term "and/or", and inserted -- at least one of the nucleic acids from (i) or (ii) --.

D. Claim 16

Claim 16 recites an alternative expression for two limitations covering two different embodiments, i.e. position "2504" is not the same as position "2649".

Applicants have deleted the term "and/or", and inserted -- or --.

E. Claims 4-7

Claims 4-7 recite the term "several" which is infinite in scope.

Applicants have amended Claim 4 to recite "at least one".

F. Claims 8-10

Claims 8-10 recite the phrase "such as", and it is unclear as to whether the limitations following the phrase are part of the claimed invention.

By amending the claim to recite "comprising", this aspect of the Examiner's rejection is thereby rendered moot.

G. Claims 7 and 19

Claims 7 and 19 recite the phrase "in particular", which is unclear as to the intended meaning.

By amending the claims to recite "comprising" language, the Examiner's rejection is thereby rendered moot.

H. Claim 1

Claim 1 recites the term "polypeptide" in lines 21 and 22, which lacks antecedent basis since elsewhere in the claim the term "protein" is used.

Claim 1, along with its dependent claims as appropriate, have been amended to recite "protein" throughout.

I. Claim 15

Claim 15 recites the term "polypeptide" in line 16, which lacks antecedent basis since elsewhere in the claim the term "protein" is used.

Claim 15 has been amended to recite "protein" throughout.

J. Claims 1, 15 and 16

Claims 1, 15 and 16 are indefinite for reciting "stringent conditions" which the Examiner believes should be defined by its chemical conditions.

Applicants direct the Examiner's attention to page 3, lines 21-26 of the specification where "stringent hybridization" is well defined and within the scope of enablement. New dependent claims (Claims 49, 50, 52 and 53) directed to this subject matter have also been added.

K. Claims 1 and 4

Claim 1 is unclear as to whether the method is intended to generate an isolated S layer protein, or a fusion protein of the same. The Examiner presumes that because Applicants refer to "peptides" in lines 21-22 of the claim that the claim is intended to cover both a protein and a fusion protein. In this case, the Examiner considers claim 4 redundant for claiming a fusion/heterologous protein.

Applicants submit that the subject matter of Claim 4 is directed to a species of protein, i.e., a fusion polypeptide, which is obtainable or derived from any one of the proteins encompassed by elements i)-iii) of Claim 1. The proteins of i)-iii) are recombinant by virtue of the method steps of claim 1, and would assuredly encompass fusion polypeptides (i.e., use of open language) so long as the criteria of the fusion polypeptide satisfies the characteristics of i)-iii).

IV. Response to Objection to the Specification

The specification is objected to under 35 U.S.C. §112, first paragraph, as failing to provide an enabling disclosure for the claimed invention.

According to the Examiner, to practice the claimed invention requires that the E. coli strain POP2135 must be known and readily available to the public or obtainable by a repeatable method set forth in the specification. Since, according to the Examiner, the specification does not provide a repeatable method for obtaining the strain and it does not appear to be readily available material, the claims are not enabled under the first paragraph of §112.

The Examiner's attention is directed to page 4, lines 1-5 in the specification, where Applicants identify the situs of deposit, i.e., DSMZ, and the deposit number for the E. coli sample. The DSMZ is an International Depository Authority established under the Budapest Treaty; therefore, for the purposes of the regulations under 37 CFR 1.803, the deposit should be recognized by the USPTO.

As further substantive proof, Applicants are filing the enclosed declaration along with copies of the deposit receipt and viability statement according to which the microorganism pop2135 has been deposited with DSMZ under the provisions of the Budapest Treaty. These documents establish that access to the biological material is already available under the terms of the Budapest Treaty.

Apart from satisfying the procedural issue raised by the Examiner, Applicants further submit that pop2135 is not essential in performing the invention, but that the method can also be carried out in other gram-negative prokaryotic host cells. An example of another bacterial strain in which the process has been successfully performed, is E coli pop2136 having ATCC No. 47049 (see, for example, the enclosed data sheet for ATCC No. 47049). On the basis of the disclosure, one skilled in the art would be fully enabled in making or using the invention with other gram-negative bacterial strains.

V. Response to Rejection of Claims 1-3, 15-17, 19, 20, 46 and 47 under 35 U.S.C. §102(b)

Claims 1-3, 15-17, 19, 20, 46 and 47 are rejected under 35 U.S.C. §102(b) as being anticipated by Kuen et al (Gene, 1994).

According to the Examiner, the present claimed invention is anticipated by Kuen, since Kuen discloses cloning and expression of an S-layer protein in a prokaryotic cell system, the nucleic acid sequence of the S-layer protein (abstract; p. 116, col. 1),

transformation of cells with a vector (p. 116, Experimental and Discussion), and the nucleotide sequence including the signal sequence of the sbs gene which encodes the S-layer protein (p. 117, col. 2; figure 2; table 1; p. 119, col. 2).

Applicants traverse for the following reasons and also wish to point out that three of the authors in the Kuen reference are inventors of the present invention.

Kuen does not describe the expression of a complete sbsA gene in E. coli. After numerous attempts to establish a genomic library in an expression vector, no full-length sbsA expression vector could be isolated. The reasons for the failed attempts at cloning presumably lay in the conception and/or performance of the experiments. Kuen explicitly points this out on page 116, right column, last paragraph. Thus, the preparation of the S-layer proteins from gram-negative bacteria by expression of the corresponding DNA in gram-negative bacteria as presently claimed, was not successful prior to the filing date for this application.

As further stated by Kuen, it was presumed that the negative results might be due to the fact that the full sbsA gene is either unstable or toxic when expressed in E. coli. With respect to the Kuen reference, Applicants have overcome this cloning problem by applying PCR cloning and sequencing strategies to isolate and determine the sequence of the missing 5' fragment of the Bs sbsA gene. Thus, while Kuen describes the sequence, the authors do not teach or suggest a method for expression of the complete sequence in a gram-negative prokaryotic host cell. The present application is the first

disclosed successful cloning strategy, and accordingly, the claimed cloning process is novel over Kuen.

VI. Response to Rejection of Claims 1-17, 19, 20, 46 and 47 under 35 U.S.C. 103(a)

Claims 1-17, 19, 20, 46 and 47 are rejected under 35 U.S.C. 103(a) as being obvious over Kuen et al (Gene, 1994) in view of Deblaere et al (W09519371).

In setting forth her case for the *prima facia* obviousness of the present claims over of Kuen in view of Deblaere, the Examiner restates her comments under the anticipation rejection, and further adds that whereas Kuen does not teach or suggest the insertion of heterologous proteins or polypeptides, Deblaere teaches a fusion polypeptide consisting essentially of the S-layer protein and a heterologous polypeptide (abstract; p. 6; claims). Deblaere discloses that the heterologous protein can be a physiologically active polypeptide such as an enzyme, a polypeptide drug, a cytokine (interferon), a foreign epitope or polypeptide immunogen, etc (p. 9). The immunogen can be an antigen of a pathogen such as a virus, bacterium, fungus, yeast or parasite (pp. 9-10).

Thus, the Examiner considers that it would have been obvious to one skilled in the art to use the methods/processes as taught by both Kuen and Deblaere with the reasonable expectation of success in obtaining a process for producing pure S-layer protein, a recombinant S-layer protein or a fusion protein comprising the S-layer protein having an insertion of a heterologous polypeptide as presently claimed.

The Examiner notes that the prior art does not specifically teach the insertion at the specific positions that are set forth in pending claim 16, however, according to the Examiner, it would have been obvious to insert a foreign DNA at any cleavage site that would still allow for the assembly of the S-layer protein.

Applicants traverse the Examiner's obviousness rejection.

For the reasons discussed above, Kuen teaches away from the subject matter of the present invention, since Kuen explicitly states that cloning the full-length sbsA gene in E. coli is not possible due to instability or toxicity. Surprisingly, Applicants have found that full-length, S-layer proteins can be obtained by the process of the present nvention.

One skilled in the art would also not arrive at the subject matter of the claimed invention by combining Kuen and Deblaere. Applicants are claiming a nonobvious expression system for a recombinant SLP and a fusion SLP polypeptide, using gram-negative bacteria, preferably E. coli, as the host cell.

Deblaere does not teach or suggest the SLP of SEQ ID No. 1 (B. stearothermophilus SLP). Deblaere concerns a method and fusion proteins, the nucleic acid of which differs from a nucleic acid of the invention by its essential features. Deblaere teaches the nucleic acid sequence for fusion proteins of an S-layer protein from Bacillus sphericus (see, for example, Claim 5 including the examples). The S-layer protein of B. sphericus shares no homology with the sbsA S-layer protein of the present invention.

Deblaere does not teach expressing the SLP in a gram-negative strain of bacteria. In fact, Deblaere teaches away from using gram-negative bacteria as host cells for expression of SLPs. Deblaere specifically states that the Bacillus strain is preferred as an expression host strain, and demonstrates by references to Tsukagoshi (page 2, lines 31- page 3, line 3), that a fusion protein comprising beta-amylase of B. stearothermophilus and SLP of B. brevis, was expressed with 15 times greater efficiency in B. brevis than in E. coli cells.

Deblaere discloses "the portion of the SLP which is present in the fusion polypeptide should generally be from a SLP of the same species as the host in which the fusion polypeptide is expressed" (page 8, lines 26-29). Significantly, Deblaere does not provide motivation to combine the disclosure with Kuen, because Deblaere teaches away from using E. coli as an expression system for expression of SLP much less that E. coli should be used for expression of fusion SLPs.

Claim 4 of the invention relates to insertions within the range encoding the S-layer protein, whereas in the case of the fusion proteins of Deblaere, the foreign peptide amount is either at the N- or C-terminus of the S-layer protein. Thus, the process of the instant invention also differs fundamentally from the method of Deblaere.

In addition, unexpected advantages are obtained with the instant claimed process. As explained in the specification (page 4, paragraph 2 continued to page 7, paragraph 1), the capacity of the S-layer protein to form a correctly folded S-layer structure is surprisingly not lost despite insertions being made to the nucleic acid. Despite the

heterologous insertions, the spatial organization of the recombinant molecule is preserved in such as way as to allow functional S-layer proteins to be formed. These heterologous proteins can be created whereby the biological activity such as enzymatic activity is preserved. Thus, the present process allows one to produce recombinant S-layer proteins containing numerous immobilized, functional foreign peptides. These advantages and possibilities are neither disclosed nor rendered obvious by Kuen or Deblaere.

Accordingly, from the above discussion, it is apparent that the process and composition claims of the present invention are both novel and nonobvious since the S-layer proteins from gram-positive cells are stable when expressed intracellularly in gram-negative cells.

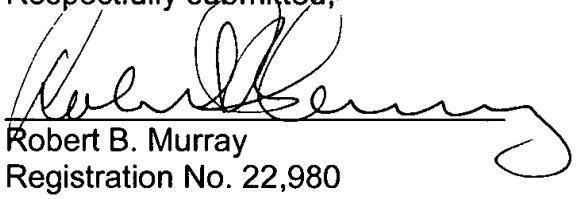
CONCLUSION

In view of the amending of the claims, the foregoing arguments and the enclosed certificate of deposit and viability statement, Applicants submit that the Examiner's rejections under §§ 102(b), 103(a) and 112, first and second paragraph, are met and overcome. The claims are now in condition for allowance, and Applicants request that the application be allowed to pass to issuance.

If for any reason the Examiner determines that the application is not now in condition for allowance, it is respectfully requested that the Examiner contact, by telephone, Applicant's undersigned attorney at the indicated telephone number to arrange for an interview to expedite the disposition of this application.

In the event this paper is not being timely filed, the applicant respectfully petitions for an appropriate extension of time. Any fees for such an extension together with any additional fees may be charged to Counsel's Deposit Account 1-2300.

Respectfully submitted,



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Page 4, first full paragraph:

"The E. coli strain [pop2125] pop2135 which was deposited on 31.01.1996 at the "Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH", Mascheroder Weg 1b, D 38124 Braunschweig under the file number DSM 10509 is particularly preferred."

Marked-up copy of original claims under 37 CFR 1.121

1. (Amended) [Process] A process for [the] production of an S-layer [proteins wherein] protein comprising

(a) transforming a gram-negative prokaryotic host cell [is provided which is transformed] with a nucleic acid [coding for] encoding an S-layer protein[which is] selected from the group consisting of

(i) a nucleic acid [which comprises the] comprising a nucleotide sequence from position 1 to 3684 [shown in] of SEQ ID NO.1[, optionally without the signal peptide-coding section],

(ii) a nucleic acid [which comprises] comprising a nucleotide sequence corresponding to the nucleic acid [from] of (i) within the scope of the degeneracy of the genetic code, and

(iii) a nucleic acid [which comprises] comprising a nucleotide sequence which hybridizes with at least one of the nucleic acids [from] of (i) [or/and] or (ii) under stringent conditions;

(b) culturing the host cell [is cultured] under conditions which [lead to an] induce expression of the nucleic acid and [to] production of the corresponding protein [polypeptide coded by it], and

(c) isolating the [resulting polypeptide is isolated] protein from the host cell.

2. (Amended) [Process] The process as claimed in claim 1, wherein the gram-negative prokaryotic host cell is an E. coli host cell [is used].

3. (Twice Amended) [Process] The process as claimed in claim 1, [wherein] comprising isolating the [polypeptide is isolated] protein from the interior of the host cell in the form of an assembled S-layer structure.

4. (Twice Amended) [Process] The process as claimed in claim 1, wherein the nucleic acid [coding for] encoding the S-layer protein [contains one or several insertions which code for] comprises at least one insertion encoding peptide or polypeptide sequences.

5. (Amended) [Process] The process as claimed in claim 4, wherein the insertions are selected from the group consisting of nucleotide sequences [which code for] encoding cysteine residues, regions with several charged amino acids or [Tyr] tyrosine residues, DNA-binding epitopes, metal-binding epitopes, immunogenic epitopes, allergenic epitopes, antigenic epitopes, streptavidin, enzymes, cytokines [or] , and antibody-binding proteins.

6. (Amended) [Process] The process as claimed in claim 5, wherein the insertions [code for] encode streptavidin.

7. (Amended) [Process] The process as claimed in claim 5, wherein the insertions [code for] encode immunogenic epitopes from a herpes [viruses , in particular herpes virus 6 or FMDV] virus.

8. (Amended) [Process] The process as claimed in claim 5, wherein the insertions [code for] encode enzymes [such as] comprising polyhydroxybutyric acid synthase or bacterial luciferase.

9. (Amended) [Process] The process as claimed in claim 5, wherein the insertions [code for] encode cytokines [such as] comprising interleukins, interferons or tumour necrosis factors.

10. (Amended) [Process] The process as claimed in claim 5, wherein the insertions [code for] encode antibody-binding proteins [such as] comprising protein A or protein G.

11. (Amended) [Process] The process as claimed in claim 5, wherein the insertions [code for] encode antigenic epitopes which bind cytokines or endotoxins.

12. (Amended) [Process] The process as claimed in claim 5, wherein the insertions [code for] encode metal-binding epitopes.

13. (Twice Amended) [Process] The process as claimed in claim 1, wherein a nucleic acid [coding for] encoding a gram-positive signal peptide is arranged in operative linkage at the 5' side of the nucleic acid [coding for] encoding the S-layer protein.

14. (Amended) [Process] The process as claimed in claim 13, wherein the nucleic acid [coding for] encoding the signal peptide comprises

(a) [the] a signal peptide-coding [section] region of the nucleotide sequence [shown in] of SEQ ID NO.1,
(b) a nucleotide sequence corresponding to the nucleotide sequence [from] of (a) within the degeneracy of the genetic code, [or/and] or
(c) a nucleotide sequence that is at least 80 % homologous to [the sequences from] at least one nucleotide sequence of (a) [or/and] or (b).

15. (Amended) [Nucleic] A nucleic acid [that codes for] encoding a recombinant S-layer protein [and is] selected from the group consisting of
(i) a nucleic acid [which comprises the] comprising a nucleotide sequence from position 1 to 3684 [shown in] of SEQ ID NO.1[, optionally without the signal peptide-coding section],
(ii) a nucleic acid [which comprises] comprising a nucleotide sequence corresponding to the nucleic acid [from] of (i) within the scope of the degeneracy of the genetic code, and
(iii) a nucleic acid [which comprises] comprising a nucleotide sequence which hybridizes with at least one of the nucleic acids from (i) [or/and] or (ii) under stringent conditions, wherein the nucleic acid contains at least one peptide or polypeptide-coding insertion within the region [coding for] encoding the S-layer protein.

16. (Amended) [Nucleic] The nucleic acid as claimed in claim 15, wherein the insertion is a site [is] located at position 582, 878, 917, 2504 [or/and] or 2649 of the nucleotide sequence [shown in] of SEQ ID NO.1.

17. (Twice Amended) [Vector, wherein it contains] A vector comprising at least one copy of a nucleic acid as claimed in claim 16.

19. (Amended) [Cell as claimed in claim 18, wherein] A transformed cell comprising a nucleic acid as claimed in claim 15 or 16 or a vector as claimed in claim17, wherein the cell is a gram-negative prokaryotic cell [and in particular an E. coli cell].

20. (Twice Amended) [Cell] A cell as claimed in claim [18] 19, [wherein it contains] comprising a recombinant S-layer structure.

46. (Amended) [Cell] A transformed cell wherein [it] the cell is transformed with a nucleic acid as claimed in claim 15.

47 (Amended) [Cell] A transformed cell wherein [it] the cell is transformed with a vector as claimed in claim 17.